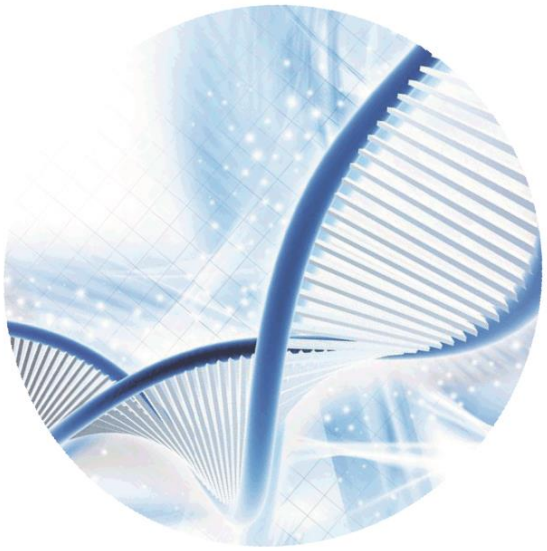


Transfection reagent

DreamFect™

Tee Technology (Triggered Endosomal Escape)
DNA Delivery

Protocol

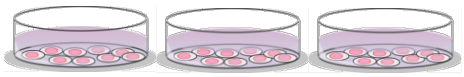
We Bring The World Of Biotechnology To You
BOCA SCIENTIFIC

OZBIOSCIENCES
The art of delivery systems

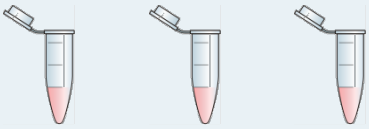
DreamFect™ Quick Protocol

To find the ideal conditions, DreamFect™ must be tested at ratios of **2 µL/µg**, **3 µL/µg** and **4 µL/µg** (µL of DreamFect / µg of DNA). For the DNA quantity, we suggest **0.125 µg** per well in 96-well, **0.5 µg** per well in 24-well and **2 µg** per well in 6-well.

1 **Seed cells to be at 70% confluent the day of transfection***



2 **Prepare 3 identical tubes of DNA**

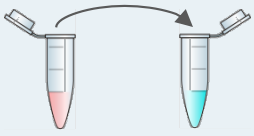


96 well plate	24 well plate	6 well plate
0.125µg in 25µL of serum-free medium or buffer* X 3	0.5µg in 50µL of serum-free medium or buffer* x 3	2µg in 100µL of serum-free medium or buffer* x 3

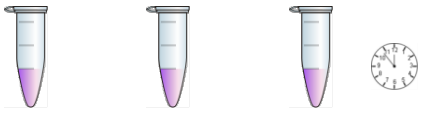
3 **Prepare 3 tubes of DreamFect™ (with 3 different amounts of reagent)**

96 well plate	24 well plate	6 well plate
0.25µL/0.375µL/0.5µL in 25µL of serum-free medium or buffer*	1µL/1.5µL/2µL in 50µL of serum-free medium or buffer*	4µL/6µL/8µL in 100µL of serum-free medium or buffer*

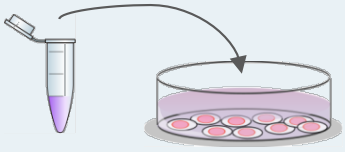
4 **Mix each tube of DNA (step 2) to each tube of DreamFect™ (step 3)**



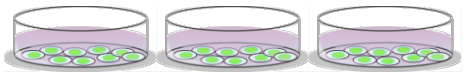
5 **Incubate 15 to 20 min at room temperature**



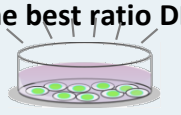
6 **Distribute each mix dropwise onto the cells to insure uniform distribution**



7 **Incubate cells for 24 to 72h at 37°C until evaluation of transgene expression***



8 **Choose the best ratio DNA:DreamFect™**



These conditions might require some further optimizations depending on your cells, DNA, RNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.

- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.

- ✓ Medium or buffer without serum & supplement must be used for the DNA/DreamFect complexes preparation. Culture medium such as MEM, DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.

- ✓ Dilute the reagent with deionized water for doses less than 1µL.

- ✓ For some cells, 24h post-transfection replace the medium with fresh pre-warm medium or just add fresh growth culture medium to the cells. In the case of cells very sensitive to transfection, the medium can be replaced after 3-4h.

Package content	DF40500: 500µL of DreamFect DF41000: 1mL of DreamFect DF45000: 5 x 1mL of DreamFect
Shipping conditions	Room Temperature
Storage conditions	Store the DreamFect transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	DreamFect is a lipopolyamine formulation specifically designed to achieve high transfection efficiency in a wide variety of cell lines and primary cells
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells preparation

It is recommended to seed or plate the cells the day prior transfection. The suitable cell density will depend on the growth rate and the conditions of the cells. Cells should not be less than 60 % confluent (percentage of growth surface covered with cells) at the time of transfection (refer to Table 1). The correct choice of optimal plating density also depends on the planned time between transfection and transgene analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Tissue Culture Dish	Adherent Cell Number	DNA Quantity (µg)	DreamFect™ Volume (µl)	Dilution Volume (µl)	Transfection Volume
96 well	0.05 – 0.2 x 10^5	0.125	0.375	2 x 25	200 µl
24 well	0.5 – 1 x 10^5	0.5	1.5	2 x 50	500 µl
6 well	2 – 5 x 10^5	2	6	2 x 100	2 mL

Table 1: Suggested cell number, DNA amount, DreamFect™ volume and transfection conditions for adherent cells

2. DNA/DreamFect complexes preparation

a. *DreamFect*: Vortex the reagent and dilute the indicated quantity of DreamFect in 25 to 100 µL of culture medium without serum and supplement (refer to Table 1).

b. *DNA*: Dilute the indicated quantity of DNA (refer to Table 1) in 25 to 100 µL of culture medium without serum and supplement.

c. Add DNA solution to DreamFect solution, mix gently by carefully pipetting up and down and incubate the mixture at room temperature for 15-20min. Do not vortex or centrifuge.

3. Transfection

a. Add the complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression.

NOTE: in case of cells very sensitive to transfection, the medium can be changed after 3-4 h or 24 h incubation with fresh medium.

IMPORTANT OBSERVATION FOR PROTEIN PRODUCTION OVER 24H

In case of protein production experiment over 24h, we recommend using two times more amounts of DNA per well to yield maximal levels of protein.

Protocol | DNA or shRNA vectors in suspension cells

1. Cells preparation

The day before transfection split the cells at a density of 2 to 5 x 10^5 cells / mL, so they are in excellent condition on the day of transfection. Incubate overnight in complete culture medium.

2. DNA/DreamFect complexes preparation

a. *DreamFect*: Vortex the reagent and dilute the indicated quantity of DreamFect in 25 to 100 µL of culture medium without serum and supplement (refer to Table 2).

Tissue Culture Dish	Suspension Cell Number	DNA Quantity (µg)	DreamFect™ Volume (µl)	Dilution Volume (µl)	Transfection Volume
96 well	0.5 – 1 x 10^5	0.5	1.5	2 x 25	200 µl
24 well	2 – 5 x 10^5	2	6	2 x 50	500 µl
6 well	10 – 20 x 10^5	4	12	2 x 100	2 mL

Table 2: Suggested cell number, DNA amount, DreamFect volume and transfection conditions for suspension cells

b. *DNA*: Dilute the indicated quantity of DNA (refer to Table 2) in 25 to 100 µL of culture medium without serum and supplement.

c. Add the DNA solution to the DreamFect solutions, mix gently by carefully pipetting up and down and incubate the mixture at room temperature for 15-20min. **Do not vortex or centrifuge.**

3. Transfection

a. While the complexes are incubating, prepare your cells in serum-free medium (or serum-containing medium) and transfer the appropriate volume to the culture dish according to Table 2. In 24-well plate for instance, plate 2x10^5 suspension cells just before transfection in 250 µL of serum free medium. Generally, serum-free condition leads to higher transfection efficiency.

b. Next, add the complexes directly onto the cells dropwise and all over the well. **IMPORTANT:** gently mix complexes with the cells by pipetting the culture medium up and down (3-4 times) to disrupt potential cell clumps and to ensure contact of the complexes with cells

c. Incubate 3 to 6h (4h is commonly used) in serum-free medium at 37°C under 5% CO2.

d. If transfections are performed in serum free medium, add serum to adjust its concentration.

e. Incubate the cells at 37°C in a CO2 incubator under standard conditions until evaluation of transgene expression. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to 72h following transfection.

f. For some cells, 24h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.

IMPORTANT OBSERVATIONS

- Transfections are optimum when performed in the absence of serum. However, transfection can also be achieved directly in the presence of serum.

- The key feature is to promote as much as you can the contact between the cells and the transfection complexes. We suggest:
    - **Option 1** - concentrate your cells: When the complexes are forming prepare your cells. Spin down the cells, resuspend them at 10 $\times 10^6$ cells / mL in serum free medium and transfer the appropriate cell number to your well according to Table 2. Thereafter, mix the complexes with the cells, incubate 15 minutes and complete the culture medium as indicated in Table 2.
    - **Option 2** - promote contact by centrifugation: Centrifuge the plate after having mixed the cells with the transfection complexes for 2-3 minutes at around 1000-1200 rpm.

- GeneBlaster™ Topaz (catalog # GB20013) can be used to boost the gene expression level in some cell lines.

Protocol | stable transfection

The same protocol can be used to produce stably transduced cells except that 48h post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48h before exposing the transduced cells to selection media.

Protocol | Co-transfection

For co-transfection of several plasmids DNA, mix the same amount of each plasmid and transfect as described above. For example, if you have two DNA plasmids, mix 0.25 µg of each plasmid, complex the 0.5 µg of DNA with 1.5 µL of Dreamfect.

Option for co-transfection

Transfections can be realized sequentially instead of simultaneously. So, cells can be transfected with one plasmid DNA first and 4h to 24h later can be transfected with the other plasmid DNA. Follow the procedure as detailed above for DNA transfection. A medium changed can be also performed between the two transfections.

Protocol | siRNA

1. Cell Preparation siRNA/DreamFect complexes preparation

The day prior transfection, prepare the cells as described in Table 1. Generally, siRNA transfection requires lower cell density than DNA transfection. The correct choice of optimal density depends on the planned time between transfection and gene knockdown analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

2. siRNA/DreamFect complexes preparation

The siRNA and DreamFect solutions should have an ambient temperature and be gently vortexed prior to use.

a. *siRNA solution*: Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (refer to Table 3) of culture medium without serum and antibiotics.

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50µL		50 µL		100 µL	
*Amount of siRNA (1 µM stock)**						
Final siRNA concentration	(µL)	(ng)	(µL)	(ng)	(µL)	(ng)
10 nM	2	27	5	67.5	20	270
20nM	4	54	10	135	40	540
50 nM	10	135	25	337.5	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

Table 3: Suggested dilution procedure and amount of siRNA to test

b. *DreamFect preparation*: Dilute 0.5 to 5 µL of DreamFect in 50 or 100µL of culture medium without serum and antibiotics (refer to Table 4 for corresponding volumes of DreamFect).

Culture vessel	96-well	24-well	6-well
Dilution serum-free medium	50 µL	50 µL	100 µL
Final transfection Volume	200 µL	500 µL	2 mL
Amount of DreamFect (µl)			
Final siRNA concentration			
10 nM	0.15	0.5	2
20nM	0.3	1	4
≥ 50 nM	0.5	2	8

Table4: Recommended amount of DreamFect per nM of siRNA used

c. Add the siRNA solution onto the DreamFect reagent. Mix gently by carefully pipetting up and down and incubate the mixture for 15 minutes at room temperature. **Do not vortex or centrifuge!**

3. Transfection

a. Add the complexes onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

b. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene knockdown analysis.

NOTES:

- Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection. We recommend 24h for RNA analysis and 48h to 72h for protein knockdown analyses.
- For some cells, 24h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.
- If cells are very sensitive to transfection, the medium can be changed after 3-4h or 24h incubation.

Optimization Protocol

We advise you to optimize your transfection conditions in order to get the best out of DreamFect™. Several parameters can be optimized:

- Ratio of DreamFect™ to nucleic acid
- Dose of nucleic acid used
- Cell type and cell density
- Culture medium composition (+/- serum)

1. DreamFect™ / DNA ratio

This is an important optimization parameter. DreamFect™ has to be used in excess compare to DNA but the optimal ratio will depend on the cell line and the vessel used. It is particularly true for 96 well plates because of adsorption processes. For optimization, first maintain a fixed quantity of DNA (according to the size of your culture dish or cell number) and then vary the ratio of DreamFect™ reagent to DNA over the suggested range in the Table 5. You can test ratios from 2 to 12 µl of DreamFect™ reagent per 1 µg DNA.

Tissue Culture Dish	DNA Quantity (µg)	DreamFect™ Volume (µL)	DreamFect™ Volume (µL) proposed interval
96 well	0.1	0.2 - 1.2	0.2 –0.4 – 0.6 – 0.8 – 1 – 1.2
24 well	0.5	1 - 6	1 – 2– 3– 4 – 5– 6
6 well	2	4 - 24	4 – 8 – 12 – 16 – 20 – 24

Table 5: Suggested range of DreamFect for optimization

2. Quantity of DNA

To achieve the optimum transfection efficiency, the amount of nucleic acid used (DNA) can be optimized. Keep the number of cells and the incubation time constant and adjust the quantity of nucleic acid while maintaining a fixed ratio of DreamFect reagent to DNA. (refer to Table 6)

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.05 – 0.4	100 µl
24 well	0.2 – 1	250 µl
6 well	0.4 – 5	1 mL

Table 6: Suggested range of DNA amounts for optimization

Following these two steps process, culture medium compositions, cell number, incubation times can also be optimized.

3. Cell number

The cells proliferating rate is also a critical parameter and the optimal confluency have to be adjusted according to the cells used. Thus, the next step is to use the optimize ratio and DNA amount obtained previously and varied the cell number to be assayed.

NOTE: The addition of the transfection complex directly to fresh seeded cells can result in a considerable increase of transfection efficiency. Significant gene expression can be detected faster with this method (in 24 hours).

4. Effect of serum /Transfection volume

Almost all cell lines transfected with DreamFect™ showed superior results if serum is present during the transfection (excepted for suspension cells). Some cell lines may behave differently and transfection efficiency can be increased without serum or under reduced serum condition. **Remember that presence of serum during complex formation is strictly prohibited, as the serum will inhibit their formation.** Transfection efficiency is attained when the initial 3-4 hours of incubation is done. Consequently, the cells may be kept in serum-free medium during the first 4 hours of transfection. If you use serum-free medium replace it by a culture medium containing serum or just add serum to the wells according to your standard culture condition after this period.
To increase the efficiency of transfection you can reduce the transfection volume suggested in Table 1 by those described in Table 6.

5. Incubation time

The optimal time range between transfection and assay for gene activity varies with cell line, promoter activity, expression product, etc. The transfection efficiency can be monitored after 24 - 72 hours by analyzing the gene product.

Additional products

- **DreamFect Stem** dedicated to stem cells transfection
- **DreamFect Gold** – for all nucleic acids transfection
- **pVectOZ Transfection plasmids (CAT, GFP, LacZ, Luciferase, SEAP)** - Positive controls and optimization of all transfection experiments

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